20-*O*-*β*-Glucopyranosyl Camptothecin from *Mostuea brunonis*: A Potential Camptothecin Pro-Drug with Improved Solubility

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Bioassay-guided fractionation of the organic extracts of whole plants of *Mostuea brunonis* (Loganiaceae), using the National Cancer Institute's (NCI) human tumor-based in vitro antitumor screen, led to the isolation and identification of camptothecin 20-*O*-*β*-D-glucoside (**1**) and three moderately cytotoxic alkaloids, the known deoxypumiloside (**2**) and strictosamide (**3**), and the new 2′-*O*-acetylstrictosamide (**4**), from the cytotoxic alkaloid fractions. While the previously unknown 20-*O*-*β*-D-glucopyranosyl camptothecin exhibited greater solubility in alcohol, DMSO−H_2O and H_2O than camptothecin, it was essentially inactive in the NCI's in vitro 60-cell line primary antitumor screen. However, it could be vulnerable to de-glucosidation in vivo, and may, therefore, merit additional evaluation as a potential prodrug of camptothecin that could be more readily formulated than the parent agent.

The present study focused upon a crude organic extract of the whole plant *Mostuea brunonis* Didr. (Loganiaceae), which showed moderate cytotoxicity in the National Cancer Institue's (NCI) 60-cell line antitumor screening panel.[1−4] Bioassay-directed fractionation led to the identification of new quinoline and indole alkaloid glycosides, 20-*O*-*β*-glucopyranosyl camptothecin (**1**) and 2′-*O*-acetylstrictosamide (**4**), respectively, as well as the biogenetically related, known alkaloids deoxypumiloside (**2**) and strictosamide (**3**). We report here the isolation, structure elucidation, and cytotoxicity of these alkaloids.

The cytotoxic crude organic extract of *M. brunonis* was initially fractionated through a solvent−solvent partition protocol.[5] The cytotoxic $CHCl_3$ fraction was then further separated by gel permeation chromatography on Sephadex LH-20, followed by HPLC on an amino-bonded phase column, to give compounds **1**−**4**.

Compound **1**, obtained as a yellow amorphous solid, gave a pseudomolecular ion at *m*/*z* 511.1698 by HRFABMS, corresponding to the molecular formula $C_{26}H_{26}N_2O_9$. A fragment ion at *m*/*z* 349 in LRCIMS, corresponding to the $[M - \text{glucose}]^+$ ion, was also observed. ^{13}C and DEPT NMR spectra showed the presence of 26 carbons, six of which were attributed to a glucosyl unit. The IR spectrum showed absorptions at 3382 cm^{-1} for hydroxyl, 1594 and 1504 cm^{-1} for aromatic, and 1732 and 1659 cm^{-1} for carbonyl functionalities. In addition to the sugar unit readily discerned from the ^1H and ^{13}C NMR data, the 1D ^1H and 2D COSY NMR spectra indicated the presence of two isolated spin systems: an *ortho*-disubstituted benzene (*δ* 8.08, 7.72, 7.88, and 8.21) and an ethyl group. There were also two aromatic proton singlets (*δ* 8.65 and 7.84) and two isolated methylenes. Apart from the glucose residue, the other signals were characteristic of the camptothecin nucleus.[6−8] The one-proton singlet at *δ* 7.84 was assigned to the olefinic proton (H-14) of the lactam D ring, while the signal at *δ* 8.65 was attributed to H-7. The two carbonyls at *δ* 171.1 and 168.3 were assignable to the lactone and lactam, respectively. Two geminally coupled doublets at *δ* 5.43 and 5.62 (J = 16.5 Hz) were assigned to the methylene protons (H-17) on ring E. HMBC relationships between the H-17 methylene (δ_H 5.43 and 5.62, δ_C 67.8) and carbons at *δ* 149.2 (C-15), 168.3 (C-16a), 171.1 (C-21) confirmed the location of the lactone and lactam carbonyls and, thus, further corroborated the camptothecin skeleton as the aglycone. The methylene protons at *δ* 5.36, which corresponded to a carbon at *δ* 51.4 in the HMQC spectrum, showed HMBC correlations to *δ* 153.9 (C-2), 133.3 (C-7), and 168.3 (C-16a) and, therefore, were attributed to H-5. The ethyl group (*δ* 0.96 for CH_3 and 2.32 for CH_2) corresponded to the side chain at C-20. The *β* configuration of the anomeric position (C-1′) was deduced from the coupling constant between H-1′ and H-2′ (J = 7.5 Hz) and the ^{13}C NMR shift of C-1′ (*δ* 101.1). The glucosyl unit was placed at C-20 on the basis of an HMBC correlation from the anomeric proton (H-1′) at *δ* 4.67 to the C-20 signal at *δ* 79.1. Thus, compound **1** was assigned as 20-*O*-*β*-D-glucopyranosyl camptothecin. HMQC and HMBC experiments allowed the complete assignment of all proton and carbon signals.

It is interesting to note that strictosamide and camptothecin analogues have not previously been isolated from the Loganiaceae. Previous studies of *Mostuea* species have yielded indole alkaloids which do not have the camptothecin type ring system.[9,10] While **1** is a new derivative, other camptothecin analogues glycosylated at C-9[11] and C-10[12,13] have been isolated from *Ophiorrhiza pumila* (Rubiaceae).

Compounds **2** and **3** were readily identified as deoxypumiloside[14,15] and strictosamide,[16−20] respectively, by comparisons of spectral and physiochemical data with literature reports. Compound **4** analyzed for $C_{28}H_{32}N_2O_9$ by HRFABMS. Its ^1H and ^{13}C NMR spectra strongly resembled those of **3**, except for additional signals for the carbonyl and methyl segments of an acetate ester. Careful comparison of the NMR data for **3** and **4** revealed that H-2′ appeared at 1.4 ppm further downfield in **4**, suggesting that C-2′ was the site of acetylation. This was confirmed by COSY, HMQC, and HMBC experiments.

Of the four alkaloids, **2**−**4** were moderately cytotoxic, but did not display any significant differential cytotoxicity;[21] the camptothecin glycoside **1** elicited only a marginal response in the NCI 60 cell line panel. Preliminary solubil-

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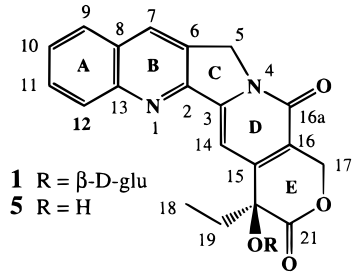

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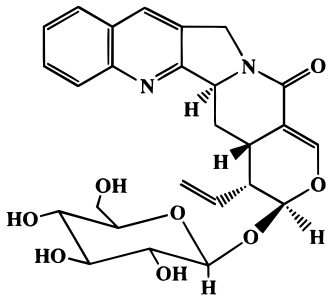
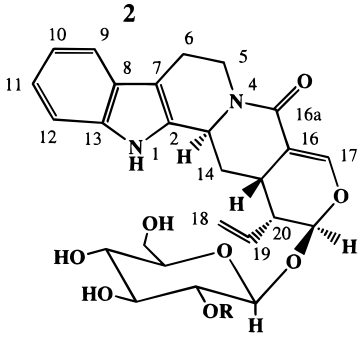
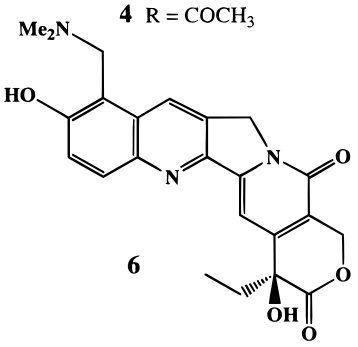
5 R = H



2



3 R = H
4 R = $COCH_3$



6

ity comparisons of **1** with camptothecin (**5**) and topotecan (**6**), however, revealed dramatic differences; 1.9 mg of **1** dissolved completely in 100 μL of H_2O (providing a clear solution), while 2 mg samples of **5** and **6** did not dissolve completely in 2 mL and 1 mL of H_2O, respectively. A topoisomerase I inhibitor, camptothecin has long been the subject of considerable preclinical work, but further development of the parent compound has been hampered by formulation problems.[22,23] The semisynthetic analogue topotecan, 9-(dimethylamino)methyl-10-hydroxycamptothecin (**6**), with improved hydrophilicity and therapeutic index, is in clinical use. Considering the improved solubility of **1** relative to camptothecin and topotecan, together with the possibility that it could serve as a source of **5** via deglucosidation in vivo, the glycoside **1** may merit further investigation for antitumor activity in vivo. While **1** is apparently not abundant in nature, the technology to prepare it readily from camptothecin does exist.

Experimental Section

Collection and Extraction. Samples of *M. brunonis* Didr. (Loganiaceae) were collected from the Lope area, south of Ayem, in central Gabon by G. McPherson in March, 1989. A voucher specimen (Q66S0056, GM 13690) was deposited at the Missouri Botantical Garden. The dried entire plants were ground (403 g), then percolated overnight at room temperature in MeOH−CH_2Cl_2 (1:1), followed by 100% MeOH. Solvents from the combined organic extracts were removed in vacuo to provide a total of 14.3 g of crude organic extract.

Isolation and Characterization. A 10.4 g portion of the organic extract was subjected to a solvent/solvent partitioning protocol[5] to yield hexane (2.11 g), CCl_4 (1.00 g), $CHCl_3$ (2.44 g), EtOAc (0.34 g), and H_2O (4.40 g) fractions. The cytotoxic $CHCl_3$ fraction (200 mg × 5) was permeated through Sephadex LH-20 (2.5 × 85 cm) with CH_2Cl_2−MeOH (1:1); six fractions were obtained. A 228.6 mg portion of the cytotoxic fraction D was subjected to HPLC on an amino column (1 × 25 cm), eluting with CH_2Cl_2−MeOH (9:1) to yield pure compounds **1** (8 mg, 0.01% dry weight), **2** (8.4 mg, 0.01%), **3** (39.0 mg, 0.06%), and **4** (7.5 mg, 0.01%).

20-*O*-β-D-Glucopyranosylcamptothecin (1): $[\alpha]_D$ +23.5° (*c* 0.52, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (4.0), 245 (3.8), 285 (3.2) 332 (3.3), 369 (3.5) nm; IR (film) ν_{max} 3382, 2928, 2356, 1732, 1659, 1594, 1504,1455, 1404, 1236, 1168, 1073, 934, 761 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 8.65 (1H, s, H-7), 8.21 (1H, d, J = 8 Hz, H-12), 8.08 (1H, d, J = 7.5, H-9), 7.88 (1H, ddd, J = 8, 8, 1, H-11), 7.84 (1H, s, H-14), 7.72 (1H, ddd, J = 8, 7.5, 1, H-10), 5.62 (1H, d, J = 16.5, H-17a), 5.43 (1H, d, J = 16.5, H-17b), 5.36 (1H, s, H-5), 4.67 (1H, d, J = 7.5, H-1′), 3.61 (1H, dd, J = 12, 11, H-6′a), 3.41 (1H, dd, J = 12, 11, H-6′b), 3.38 (1H, dd, J = 8, 8, H-2′), 3.33 (H, m, H-5′), 3.24 (1H, dd, J = 8.5, 8, H-4′), 3.04 (1H, dddd, J = 8.5, 8, 5.5, 2.5, H-3′), 2.32 (1H, dq, J = 21.5, 8, H-19a), 2.17 (1H, dq, J = 21.5, 8, H-19b), 0.96 (1H, t, J = 8, H-18); ^{13}C NMR (125 MHz, CD_3OD) δ 171.1 (C-21), 168.3 (C-16a), 153.9 (C-2), 149.6 (C-13), 149.2 (C-15), 146.2 (C-3), 133.3 (C-7), 131.9 (C-11), 130.9 (C-6), 129.9 (C-8), 129.8 (C-12), 129.7 (C-9), 129.1 (C-10), 122.0 (C-16), 101.5 (C-14), 101.1 (C-1′), 79.1 (C-20), 78.2 (C-5′), 78.1 (C-3′), 75.6 (C-2′), 71.1 (C-4′), 67.8 (C-17), 62.2 (C-6′), 51.4 (C-5), 33.9 (C-19), 8.4 (C-18); HMBC, δ_c (correlations to δ_H) 171.1 (H-19a, H-19b, H-17a, H-17b), 153.9 (H-7, H-14, H-5), 149.6 (H-7, H-9, H-11), 149.2 (H-17, H-19), 146.2 (H-14), 133.3 (H-9), 131.9 (H-12) 130.9 (H-5), 122.0 (H-14), 101.1 (H-2′), 79.1 (H-1′, H-18, H-19), 71.1 (H-5′, H-6a′); HRFABMS *m/z* 511.1698, ($[MH]^+$, calcd for $C_{26}H_{27}N_2O_9$, 511.1717); LRCIMS *m/z* 511 $[MH]^+$ (7), 460 (22), 443 (3), 349 (6), 307 (62), 289 (57), 155 (100), 139 (99), 107 (56), 89 (45), 77 (42).

2′-*O*-Acetylstrictosamide (4): $[\alpha]_D$ −63° (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (4.5), 313 (3.2), 320 (3.2) nm, 370 (3.2), 378 (3.2); IR (film) ν_{max} 3356, 2921, 1732, 1651, 1587, 1463, 1434, 1303, 1236, 1190, 1075, 894, 830, 745 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 7.37 (1H, d, J = 8 Hz, H-9), 7.35 (1H, s, H-17), 7.33 (1H, d, J = 8, H-12), 7.07 (1H, ddd, J = 8, 8, 1, H-11), 6.98 (1H, ddd, J = 8, 8, 1, H-10), 5.63 (1H, dt, J = 10, 17, H-19), 5.40 (1H, d, J = 2, H-21), 5.37 (1H, dd, J = 2, 17, H-18a), 5.32 (1H, dd, J = 2, 10, H-18b), 5.07 (1H, dd, J = 2, 5, H-3), 4.93 (1H, dt, J = 5, 13, H-5a), 4.68 (1H, d, J = 8, H-1′), 4.44 (1H, dd, J = 8, 8, H-2′), 3.86 (1H, dd, J = 2, 12, H-6′a), 3.63 (1H, dd, J = 5, 12, H-6′b), 3.41 (1H, dd, J = 8, 8, H-3′), 3.33 (1H, m, H-5′), 3.24 (1H, dd, J = 8, 8, H-4′), 3.15 (1H, dt, J = 5, 13, H-5b), 2.95 (1H, m, H-6a), 2.70 (1H, m, H-6b), 2.62 (1H, m, H-15), 2.59 (1H, m, H-20), 2.45 (1H, ddd, J = 2, 5, 14, H-14β), 2.01 (1H, ddd, J = 2, 5, 14, H-14α), 1.20 (3H, s, $-CH_3$); ^{13}C NMR (125 MHz, CD_3OD) δ 171.0 ($COCH_3$), 166.9 (C-16a), 148.4 (C-17), 137.9 (C-13), 134.6 (C-2), 133.7 (C-19), 128.6 (C-8), 122.3 (C-11), 120.6 (C-18), 119.9 (C-10), 118.4 (C-9), 112.4 (C-12), 110.1 (C-7), 109.9 (C-16), 96.2 (C-1′), 96.1 (C-21), 78.4 (C-5′), 75.3 (C-3′), 74.1 (C-2′), 71.5 (C-4′), 62.4 (C-6′), 55.2 (C-3), 45.0 (C-5), 44.0 (C-20), 26.9 (C-14), 25.1 (C-15), 22.0 (C-6), 19.7 (CH_3); HRFABMS *m/z* 541.2184 ($[MH]^+$, calcd for $C_{28}H_{33}N_2O_9$, 541.2186); LRCIMS *m/z* 541 $[MH]^+$ (10), 540 $[M^+]$ (8), 522, (4), 482 (12), 460 (7), 444 (20), 422 (18), 397

(9), 337 (4), 329 (49), 307 (66), 269 (39), 176 (54), 154 (72), 137 (100), 119 (58), 85 (57).

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